2925-Pos Board B695
Does Aspartate112 Mutation Convert the Human Voltage Gated Proton Channel into a Hydroxide Channel?
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Voltage gated proton channels (H_V1) are the most selective channels known, with no detectable permeability to any ion besides H^+. We recently identified the selectivity filter of the human voltage gated proton channel (hH_V1). Mutation of an aspartate residue, Asp^{112}, in the middle of the S1 transmembrane domain resulted in loss of proton specificity. Surprisingly, mutant channels were anion selective. Cation substitution did not affect V_{rev} at all. Replacing $CH_3SO_3^-$ by Cl^- shifted V_{rev} negatively, showing that that Cl^- is more permeable than $CH_3SO_3^-$. Dilution of the bath solution by 90% with isotonic sucrose does not change pH (or pOH), but shifted the measured reversal potential positively, in both Cl^- and $CH_3SO_3^-$ bath solutions, indicating anion selectivity. However, although 10-fold reduction in ionic strength produced large V_{rev} (reversal potential) shifts of up to +40 mV, it did not shift V_{rev} by +57 mV, as expected for pure anion selectivity. The sub-Nernstian shift means that the mutant channels must be permeable to either H^+ or OH^-, the only ions whose concentration does not change. Because E_H and E_{OH} are identical, it is difficult to distinguish these species. We will present and discuss several types of evidence, most of which suggests that OH^- and not H^+ is permeant in mutant hH_V1 channels.

2926-Pos Board B696
Ground-State Proton Transfer in Green Fluorescent Protein Measured by NMR
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Proton transfer plays an important role in the optical properties of fluorescent proteins. Excited-state proton transfer (ESPT) is responsible for wtGFP's anomalously large Stokes shift and a number of FPs have found use as *in vivo* pH indicators. Unlike ESPT, ground-state proton transfer cannot be synchronously initiated (i.e. with an excitation pulse) and must be studied more indirectly. We report an NMR method in which GFP is labeled with a ^{13}C at the position adjacent the phenol hydroxyl on the chromophore. This position is a highly specific and sensitive reporter of the ionization state as measured by direct-detect ^{13}C-NMR. Through lineshape analysis and time-resolved NMR spectroscopy we obtain kinetic parameters for proton transfer. We find that GFPs having internal proton transfer and capable of ESPT show rapid interconversion of the protonated and deprotonated states (on the order of microseconds) while GFPs which must transfer protons externally to the solvent have much slower equilibration (on the order of milliseconds). Parallel measurements with less direct fluorescence correlation spectroscopy reveal interesting discrepancies perhaps suggestive of light-driven structural dynamics.

2927-Pos Board B697
The Spatial Mapping of the Metabolic Cofactor NADH within Live Progenitor Stem Cells
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NADH is a naturally occurring bi-product and regulatory metabolite associated with cellular respiration. The quantification using the difference lifetime of autofluorescence of free and bound NADH has the potential to enhance the understanding of a range of cellular processes including apoptosis, cancer pathology and enzyme kinetics. Fluorescence lifetime imaging microscopy (FLIM) enables not only examination of the spatial location of the cofactor within live cells but also of its state.
Here we describe the use of phasor FLIM to spatially map the fluorescence lifetimes of NADH in both free and bound form within live undifferentiated myoblast cells. The phasor approach graphically depicts the change in lifetime at a pixel level without the requirement for fitting the decay. The phasor representation enables the possibility for a direct comparison of either optical sections (i.e. different focal planes) of one cell or multiple cells to enable a global analysis. A comparison of myoblast cells induced to differentiate through serum starvation and undifferentiated cells show differing spatial distribution of the different forms of NADH. Cells due to undergo differentiation displayed a short lifetime representing free NADH situated around the cytoplasmic periphery and a longer lifetime attributed to the presence of bound NADH just outside of the nucleus. Differentiated cells displayed redirection of the distribution of free NADH located mainly within the nucleus while the bound form remained directly comparable to that of the other cells. Furthermore, there appears to be a spatial shift in the distribution of lifetimes at a pixel level within the phasor plot. We show that the states of differentiation of myocytes may be determined through the phasor FLIM analysis of the autofluorescent properties of NADH.

2928-Pos Board B698
On the Mechanism of Synergistic Cytotoxicity of Vitamins C and K_3: Experiments *in Vitro* and Quantum-Chemical Analysis
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Most patients with hepatocellular carcinoma are inoperable and hepatoma cells are resistant to conventional chemotherapies. So, it is important to develop novel therapies. Treatment of mouse hepatoma MH-22A cells by vitamins C and K_3 at the ratio of 100:1 greatly enhanced their cytotoxicity. When cells were subjected to vitamin C at 200 μM or K_3 at 2 μM separately, viability was more than 90%. However, when vitamins C and K_3 were combined at these concentrations, less than 10% of cells survived.
To elucidate the mechanism of this synergy, theoretical quantum-chemical analysis of the dynamics of intermolecular electron transfer (IET) processes within the complexes containing C and K_3 was carried out. Optimization of the ground state complex geometry was provided by means of GAUSSIAN03 package. Simulation of the IET was done using NUVOLA package, in the framework of molecular orbitals. Rate of IET was calculated using Fermi Golden rule.
Two concurrent pathways of a plausible mechanism of the synergistic action of vitamins C and K_3 were analyzed: increasing of acidity in the near surrounding of complex significantly affects redox-cycling and creating the most stable [C2K] complex stabilizes acidity at a high level.
Out of the complexes of vitamin K_3 with five different forms of vitamin C, the most stable one was the complex with de-hydro-ascorbic acid [C2K], and the second one - with ascorbic acid [C1K]. The spectra of IET represent the different nature of complexes. There are several possibilities for electron transfer in the less stable [C1K] complex, while for the most stable [C2K] complex; the number of resonant states significantly decreases. This confirms that the creation of complex [C1K] escalates increasing of acidity.

Molecular Mechanics & Force Spectroscopy II

2929-Pos Board B699
Single-Molecule Rupture Dynamics on Multidimensional Landscapes
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The unfolding of a biomolecule by stretching force is commonly treated theoretically as one-dimensional dynamics along the reaction coordinate coincident with the direction of pulling. Here we explore a situation, particularly relevant to complex biomolecules, when the pulling direction alone is not an adequate reaction coordinate for the unfolding or rupture process. We show that in this case the system can respond to pulling force in unusual ways. Our theory points out a remarkably simple, but largely overlooked, mechanism of the complex responses of biomolecules to force. The mechanism originates from the basic property of the transition state to change its structure under applied force. A relationship is established between a key experimental observable, force-dependent lifetime, and the microscopic properties of the biomolecule in the form of an analytical solution to the problem of a force-induced molecular transition in two dimensions. The theory is applicable to biological contexts ranging from protein folding to ligand-receptor interactions.

2930-Pos Board B700
Mechanical Features of Transthyretin: Probing Folding Intermediates of an Amyloid Forming Protein by Single Molecule Force Spectroscopy
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Formation of amyloid aggregates by transthyretin (TTR) is associated with severe neurological disorders. Over 80 naturally-occurring mutations have been reported to promote the misfolding of TTR leading to an enhanced aggregation propensity. Yet, such dramatic decrease in stability is generally not apparent from analysis of their corresponding X-ray crystal structures.